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14. ABSTRACT
We have observed a dramatic increase of adipocyte-derived matrix protein collagen VI (COL6) level during cancer progression, particularly it relates to a discrete C-terminal domain of the alpha3 subunit of COL6. I have established 2 lines of transgenic mice which overproduce C-terminal domain, called "C5", of the COL6A3 (COL6A3-C5) under the control of MMTV promoter and crossed with MMTV-PyMT mice to see the C5 effects on mammary tumor progression in vivo. Our results indicate that COL6A3-C5 augments primary tumor growth and pulmonary metastasis in MMTV-PyMT mammary tumor mice model in vivo. Based on the cDNA microarray data with tumor tissues, COL6A3-C5 seems to be a signaling molecule regulating a kinase (or a phosphatase) activity which may be through a specific receptors that remains to be identified. Furthermore, treatment with COL6A3-C5 neutralizing antibodies in MMTV-PyMT mice protects mammary tumor progression. I have determined the effects of COL6 on the TZD mediated enhancement of cisplatin susceptibility with PyMT/COL6-/- mice. Tumor growth in PyMT/COL6-/- mice was significantly attenuated either by cisplatin or by a combination of cisplatin and TZD compared to PyMT/COL6+/+. To better understand the COL6 effects on drug resistance, I have tested COL6A3-C5 neutralizing antibodies in combination with cisplatin and TZD using the primary cultured tumor cells implantation system. To visualize the tumor progression in vivo, I generated MMTV-F635 transgenic mice which overproduce an infrared fluorescence protein specifically in the mammary epithelial cells. This allows me for the first time to monitor efficacy of treatment modalities longitudinally in mice.

15. SUBJECT TERMS
Tumor microenvironment, adipocyte, ECM, collagen VI, breast cancer, cisplatin resistance, TZD

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INTRODUCTION

Subject:

The interactions between malignant ductal epithelial cells and the surrounding stromal cells play a crucial role in mammary tumor progression (*1*). The adipocyte is one of the predominant stromal cell types in the tumor microenvironment. The adipocyte is a highly active endocrine cell secreting numerous signaling molecules and profoundly shaping stromal-epithelial interactions (*2-3*). However, it remains unclear which specific adipocyte-derived factors are involved and how malignant cells are regulated by these factors *in vivo*. We have previously identified a prominent adipocyte-derived extracellular matrix (ECM) protein, type 6 Collagen (COL6), as an important stimulator of mammary tumor growth. It is highly up regulated in human breast cancer patients (*4-5*). Our previous work of COL6 knock-out (KO) mice in the background of MMTV-PyMT (mammary tumor virus-polyoma middle T antigen) demonstrated significantly attenuated rates of early hyperplasia and primary tumor formation (*5*).

Purpose:

In this project, I have focused on the carboxy-terminal domain of COL6 alpha3 subunit named COL6A3-C5, because I previously determined that the carboxy-terminal domain of COL6 is highly enriched in malignant tumor tissues relative to full length COL6. The COL6A3-C5 domain is cleaved off from the COL6 microfilament ECM structure upon secretion from the adipocyte (*6*). We believe that the adipocyte-derived, cleaved form of the C5 protein acts as a signaling molecule, influencing tumor growth and metastasis through various downstream signaling pathways. Therefore, I have explored the roles of **adipocyte-derived COL6A3-C5 as a novel mitogen** in mammary tumor progression *in vivo*. Furthermore, it has been suggested that increased levels of COL6 are involved in chemo-resistance to platinum-based therapeutic approaches in cancers, which is a widely used chemotherapeutic agent (*7-8*). The molecular mechanisms that explain platinum resistance in tumor cells remain largely unknown. Anti-diabetic agents, thiazolidinediones ("TZD's", PPARγ agonists) have been studied in human cancer therapy based on anti-mitogenic and terminal differentiation therapy as well as combination therapy with platinum-based regimen (*9-10*). Our observation that COL6 level is decreased by TZD treatment let us to investigate whether **COL6 is involved in the synergistic effects of TZD in combination with platinum-based therapies *in vivo*.**

Scope of research:

Here, I highlight the adipocyte-derived extracellular matrix as an important and novel site of modulation of cancer cell behavior within the tumor microenvironment. Contributions of stromal adipocytes on cancer cell behavior are expected to have more relevance to obesity-related cancers, such as colon, renal, pancreas, and post-menopausal breast cancer. My studies highlight novel mechanisms linking obesity and aggressive cancer progression and provide therapeutic strategies for these obesity-related cancers.

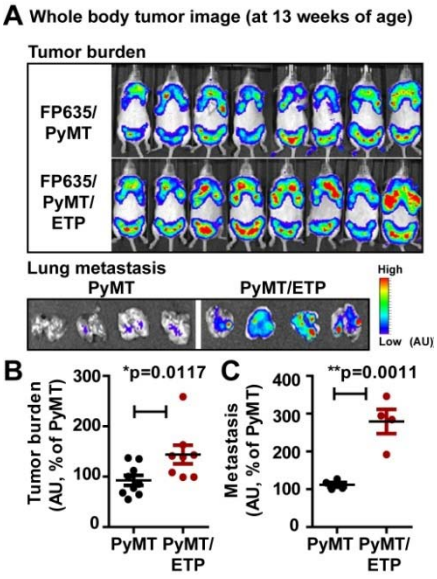
BODY

***Specific Aim1*. Analyze the phenotype of MMTV-COL6A3-C5 transgenic mouse in the background of MMTV-PyMT breast cancer model.**

Task 1. Determine the pro-mitotic activities of COL6A3-C5 in mammary cancer cells (1-9 months): ***Completed in year 1***
Task 2. Characterization of the VIa3-C5 transgenic mouse model in the MMTV-PyMT background (1-24months): ***Completed in year 1***

Development of in vivo imaging tools to quantify tumor volume: ***Completed in year 2***

The detailed quantification of lesion size remains a challenge. If the differences in lesion growth are visually apparent, the appropriate quantification is fairly straightforward. For cases in which the differences are more subtle, I established new protocols for volume integration of lesions using an infrared scanner. I have generated the MMTV-infrared fluorescence transgenic mice, which allow us to visualize breast tumor progression in whole animals with the IVIS scanner (UTSW Cancer Imaging Center). I bred MMTV-F635 mice with PyMT/COL6A3-C5 and PyMT/COL6$^{-/-}$ mice to quantify tumor volume at the whole animal level.



Notably, I shall refer to COL6A3-C5 as "Endotrophin (ETP)" here after.

Figure 1. COL6A3-C5 (ETP) augments primary tumor growth and pulmonary metastasis in the background of MMTV-PyMT mice. Tumor volumes for 13-week-old FP635/PyMT and FP635/PyMT/ETP mice was monitored with a fluorescence scanner (IVIS, Caliper life science). Metastatic burden was determined by fluorescence signals in lung tissues. **A**. Representative whole body images for tumor burden. Quantified results for tumor burden (**B**) and metastasis (**C**) are represented as mean ± SEM (n=8-9/ group). *p=0.0117, **p=0.0011 vs. PyMT by *unpaired t-test*.

***Specific Aim2*. Generation of polyclonal antibodies and monoclonal antibodies that can neutralize the activity of the collagen VI*α*3-C5 domain, the region of the protein that we believe to confer resistance to cisplatinum through induction of metallothioneins**

Task 1. Generate VIa3-C5 specific polyclonal antibodies (1-12 months): ***completed in year 1***
Task 2. Generation of VIa3-C5 specific monoclonal antibodies (1-12 months): ***completed in year 1***
Task 3. Verify the neutralizing activities of VIα3-C5 monoclonal antibodies (1-12 months): ***completed in year 1***

***Specific Aim3*. Compare the susceptibility to platinum-based cancer regimens in wild-type vs. collagen VI null mice (COL6$^{-/-}$) in the MMTV-PyMT (6-24 months).**

Task 1. Generate collagen VI null mice and breed with MMTV-PyMT mice (6-12 months): ***Completed in year 1***

Task 2. Characterize the collagen VI effects on cisplatin resistance in MMTV-PyMT mice model (9-24 months): ***Completed in year 3***

2a. Evaluated cisplatin drug toxicity and set up the optimal protocol of cisplatin treatment in wild-type and collagen VI null mice in the MMTV-PyMT background (9-18 months)

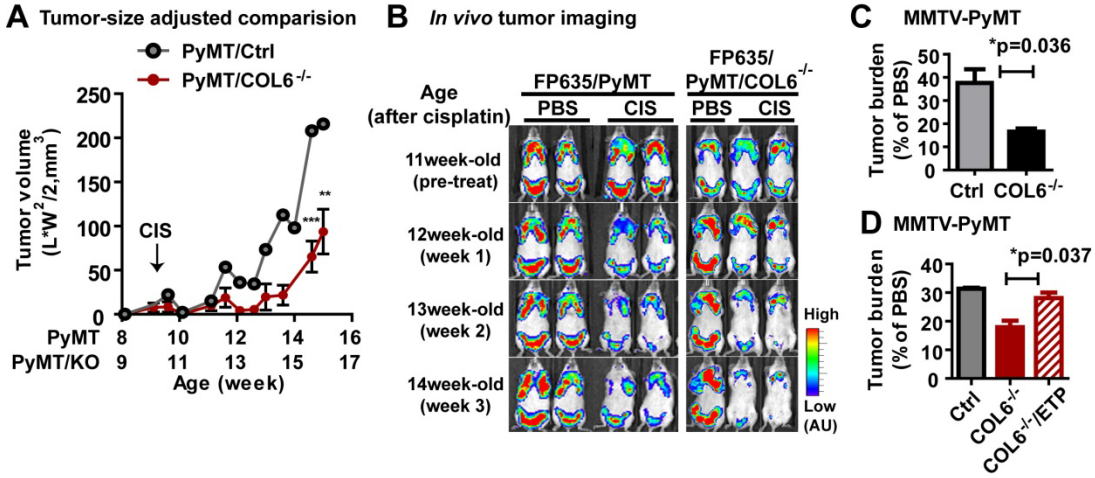


Figure 2. The absence of COL6 in PyMT mice sensitizes tumors to cisplatin treatment. A. Cisplatin (1mg/kg, ip, 3 times/week) was given to 9 week old PyMT mice and 10 old PyMT/COL6$^{-/-}$ mice (tumor size adjusted) over the course of tumor progression. Tumor volume was determined by caliper measurements. Data represent mean ± SEM (n=5-7/ group). **p<0.01, ***p<0.001 vs. PyMT/CIS by *2-way ANOVA*. **B-C**. 11 week old FP635/PyMT and FP635/PyMT/COL6$^{-/-}$ mice were given cisplatin (1 mg/ kg, ip, 2 times/ week) or PBS over the course of tumor progression. Tumor burden at a whole body level was monitored with a fluorescence scanner (IVIS) once a week. Representative images (**B**) and quantification (**C**) showing increased cisplatin sensitivity in PyMT/COL6$^{-/-}$ mice. Tumor burden at the end point was determined and represented as mean ± SEM (n=5/ group). *p=0.036 vs. FP635/PyMT/CIS by *unpaired student t-test*. **D**. 11 weeks old FP635/PyMT/COL6$^{-/-}$ mice and FP635/PyMT/COL6$^{-/-}$ /ENDOTROPHIN mice were given cisplatin for 1-month compared to PyMT control littermates. Tumor burden was determined by fluorescence signal intensity. Data represent mean ± SEM (n=5/ group). *p=0.037 vs. FP635/PyMT/COL6$^{-/-}$ by *unpaired student t-test*.

2b. Tumor tissues or MET cells (mammary epithelial tumor cells) from PyMT/COL6$^{+/+}$ vs. PyMT/COL6$^{-/-}$ mice were be implanted into WT mice. Cisplatin treatment was performed on the tumor bearing mice when the tumor size reached 5 mm diameter and treatment was ceased when the tumor regression was apparent. (12-24 months)

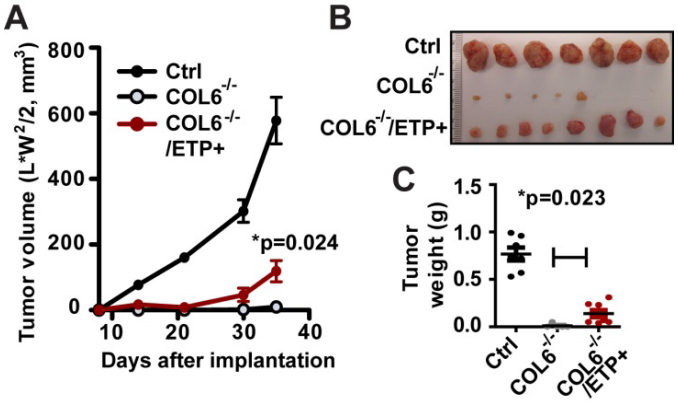


Figure 3. Endotrophin reconstitution into COL6$^{-/-}$ cancer cells rescues tumor growth. Indicated cancer cells isolated from the PyMT, PyMT/COL6$^{-/-}$ and PyMT/COL6$^{-/-}$/ETP mice were mixed with the same volume of matrigel (50 µl) and implanted into WT mice to monitor tumor growth (0.5 × 10^6 cells/ mouse). **A.** Tumor growth was determined by caliper measurements. Quantified results represent means ± SEM (n=5-8/ group). *p=0.024 vs. KO by *unpaired t-test*. **B**. A representative image was acquired 35 days post-implantation. **C**. Tumor weight was determined and represented as mean ± SEM (n=5-8/ group). *p=0.023 vs. KO by *unpaired t-test*.

Primary cultured mammary epithelial cancer cells isolated from tumor tissues of PyMT/COL6$^{+/+}$ vs. PyMT/COL6$^{-/-}$ mice were implanted into FVB wild-type mice and monitored tumor growth. COL6$^{-/-}$

cancer cells fail to thrive in the wild-type host (**Fig. 3**). Therefore, I tested cisplatin sensitivity in COL6$^{-/-}$ cancer cells with allograft models. Nevertheless, reconstitution of endotrophin into COL6$^{-/-}$ mice partly recovered the tumor growth, implying that endotrophin is a potent tumor-promoting factor. Furthermore, endotrophin *per se* confers cisplatin resistance in PyMT/COL6$^{-/-}$ as seen in **Fig. 2D**. In light of these results, I started to focus my analysis mostly on endotrophin that we identified as a tumor-promoting factor.

Furthermore, endotrophin confers cisplatin resistance in PyMT mice model, suggesting that the levels of endotrophin are directly associated with chemo-responsiveness (**Fig. 4**).

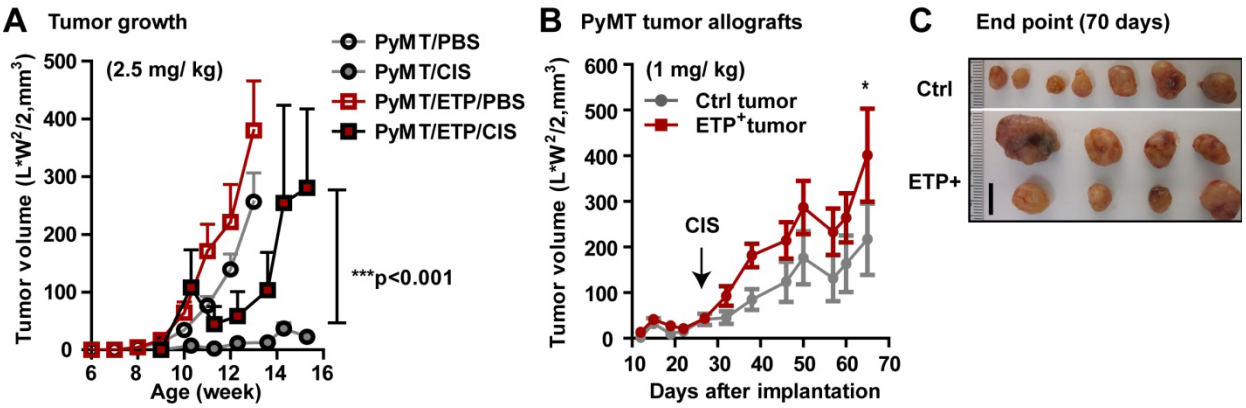


Figure 4. ENDOTROPHIN overexpression confers cisplatin resistance in PyMT mice. **A**. 10-weeks-aged PyMT and PyMT/ENDOTROPHIN mice were given high dosage of cisplatin (2.5 mg/ kg, ip, 2 times/ week). Tumor growth was determined by caliper measurements. Data represent mean ± SEM (n=7-10/ group). $^{***}p<0.001$, PyMT/CIS vs. PyMT/ENDOTROPHIN/CIS by *2-way ANOVA*. **B-C**. A piece of tumors taken from PyMT (Ctrl-tumor) and PyMT/ENDOTROPHIN (endotrophin^{+}-tumor) mice were implantation of into isogenic wild-type hosts. Cisplatin (1mg/ kg, ip, 2 times/week) were injected at 3-weeks post implantation for tumor progression. Tumor volume was determined by caliper measurement. Quantification (**B**) and representative images (**C**) showing increased cisplatin resistance in ENDOTROPHIN^{+}-tumors. Data represent mean ± SEM (n=7-8/ group). $^{*}p<0.05$ vs. Ctrl-tumors by *2-way ANOVA*. Representative images were taken at 70-days post implantation. Scale: 10 mm.

2c. Treat experimental cohorts with cisplatin and measure tumor volumes, rates of apoptosis, and expression levels of metallothioneins (MTs) (18-24 months). To obtain quantitative results with significance, I performed these experiments in triplicates using independent cohorts: ***completed in year2***

Specific Aim4. **Examine the roles of collagen VI on synergistic effects between thiazolidinediones (TZDs) and cisplatin therapy (3-36 months):** ***Completed in year 3***

Task 1. Examine the regulation of VIα3-C5 by TZDs treatment *in vivo* (3-8 months)
1a. Obtain experimental cohorts (3-6 months): At least 5 mice will be used in the experiments.
1b. Perform immunohistochemistry (IHC) with histology samples of mammary gland tissues of MMTV-PyMT mice with or without TZD treatments for 2 to 4 weeks (4-8 months)

Task 2. Compare cisplatin susceptibility in collagen VI null and wild type mice in the background of MMTV-PyMT mice with or without TZDs treatments (6-24 months)
2a. Get experiment cohorts (6-12 months): 7-10 mice in each group will be used in the experiments.
2b. Optimize the protocols of cisplatin treatment in combination with TZD as mentioned in Specific Aim3- Task 2 (12-18 months)
2c. Perform cohort experiments (18-24 months): Collect samples and analyze it.

Tumor growth was efficiently reduced in PyMT mice given TZDs (20 mg/kg) in combination with cisplatin (1 mg/kg) compared to those given only cisplatin (**Fig. 5A)**. To see whether COL6 is

involved in the synergistic effects of TZD on platinum-based therapy, I determined the expression levels for COL6 in response to chemotherapy. The mRNA levels of COL6A3 in tumor tissues of PyMT mice were significantly increased in response to cisplatin treatment; this increase was dramatically suppressed by combination with TZDs (**Fig. 5B**). These results indicate that COL6A3 levels may have an impact on the degree of synergy between TZDs and chemotherapy *in vivo*. Nevertheless, whether and how COL6A3 directly contributes to drug responsiveness is not known.

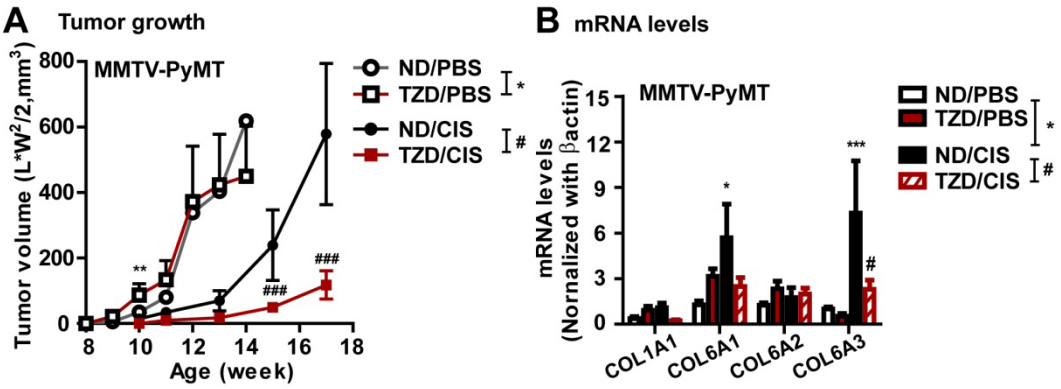
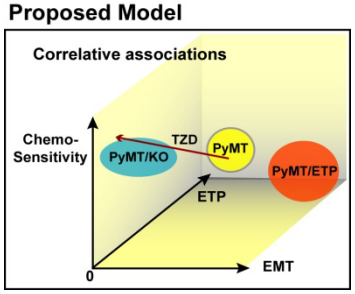


Figure 5. TZD augments cisplatin sensitivity and correlates with the COL6A3 levels. A. PyMT mice were given TZD containing chow (supply approx. 20mg/kg/day TZD) or normal-diet (ND) starting at 8-weeks of age, and cisplatin (1mg/kg) or PBS treatment was initiated at 10 weeks of age (i.p., 3 times/week) over the course of tumor progression. Tumor volumes represents mean ± SEM (n=7/ group). **p<0.01 ND/PBS vs. TZD/PBS; $^{###}$p<0.001 ND/CIS vs. TZD/CIS by *2-way ANOVA*. **B**. Total RNA was extracted from tumor tissues in each group. mRNA levels for COL1A1, COL6A1, -A2, -A3 were determined by qRT-PCR and normalized with β-actin. Quantitative results represent mean ± SEM (n=7/ group). *p<0.05, ***p<0.001 ND/PBS vs. ND/CIS; $^{\#}$p<0.05 ND/CIS vs. TZD/CIS by *2-way ANOVA.*

Histologically, I observed that necrotic lesion areas caused by cisplatin were decreased by endotrophin overexpression, whereas these lesions were significantly increased by combining the cisplatin treatment genetically with a COL6$^{-/-}$ mutation or pharmacologically with TZD treatment (**Fig. 6A-B**). As such, endotrophin levels were dramatically reduced in tumor tissues of both COL6$^{-/-}$ and TZD treatment groups compared to controls (**Fig. 6C-D**). Immunostaining for EMT markers that include either the loss of E-cadherin or an increase in vimentin expression, showed a significant increase of EMT following cisplatin treatment in tumor tissues (**Fig. 6E-H, PBS vs. CIS)**. Moreover, this increase was further augmented in endotrophin-transgenic mice (**Fig. 6E-H, CIS vs. endotrophin/CIS**), suggesting that the endotrophin-induced EMT at least partly accounts for the cisplatin resistance seen in endotrophin-transgenic mice. In accordance with this, EMT as judged by immunostaining for vimentin, a mesenchymal cell marker, was significantly suppressed in the COL6$^{-/-}$ and TZD groups (**Fig. 6E-F)**. The acquisition of mesenchymal cell-like traits in EMT process was therefore profoundly suppressed by either COL6$^{-/-}$ or TZD combination. Notably, the loss of the epithelial cell marker E-cadherin in both groups was comparable to those seen in cisplatin monotherapy (**Fig. 6G-H**). This may be because cisplatin treatment affects the plasma membrane integrity of epithelial cancer cells. Collectively, these results suggest that modulation of endotrophin levels either genetically or pharmacollogically with COL6$^{-/-}$ mice or TZDs, respectively, was tightly associated with EMT levels in tumor tissues, and this correlative decrease of endotrophin and EMT at least partly accounts for the increased cisplatin sensitivity observed in the COL6$^{-/-}$ or TZDs combination groups (see **Proposed Model**).

Proposed Model



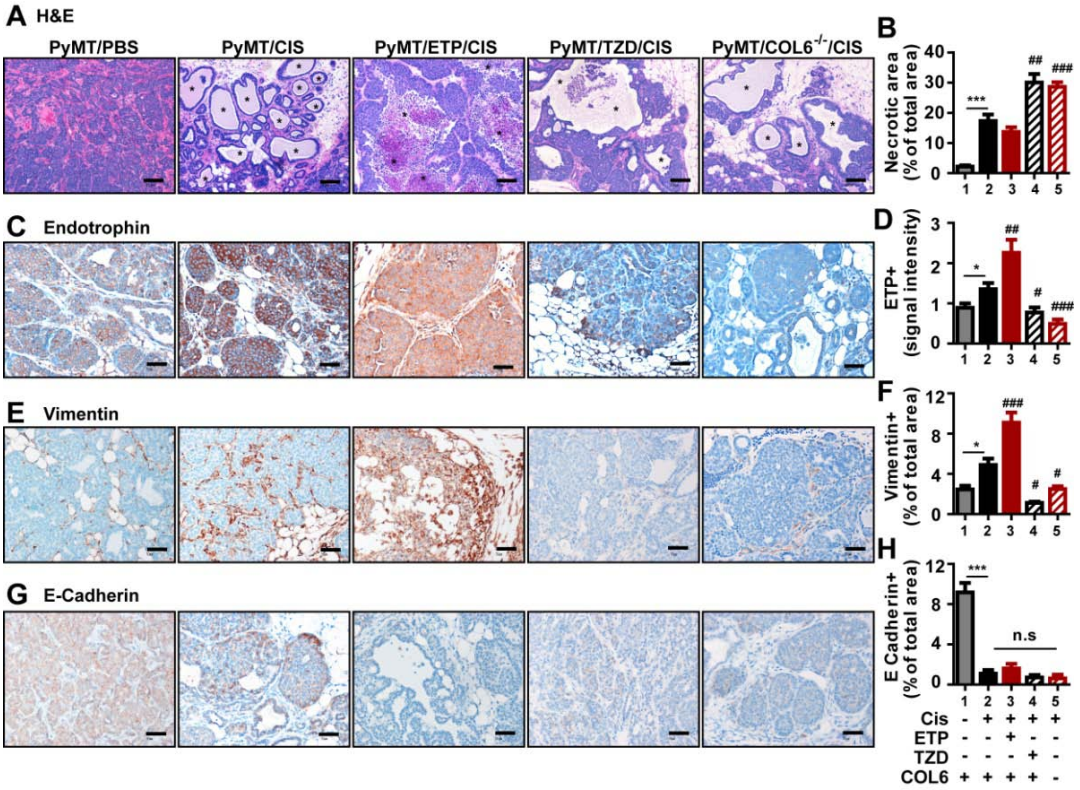


Figure 6. Histological analysis of tumors in PyMT mice with different levels of ENDOTROPHIN after chemotherapy. A-B. H&E staining (**A**) and necrotic lesion area quantification (**B**) on tumors, showing decreased cisplatin sensitivity in PyMT/ENDOTROPHIN mice, whereas it was increased in PyMT/TZD and PyMT/COL6$^{-/-}$ mice after cisplatin exposure. **C-D**. Endotrophin staining (**C**) and quantification (**D**), showing increased endotrophin levels upon cisplatin treatment which was further augmented in PyMT/ENDOTROPHIN mice, whereas it was barely detectable in PyMT/TZD and PyMT/COL6$^{-/-}$ mice. **E-F**. Vimentin staining (**E**) and quantification (**F**), showing increased EMT in PyMT/ENDOTROPHIN mice, whereas it was undetectable in PyMT/TZD and PyMT/COL6$^{-/-}$ mice. **G-H**. E-cadherin staining (**G**) and quantification (**H**), showing decreased membrane integrity of epithelial cancer cells after cisplatin treatment in PyMT mice. Quantified results represent mean ± SEM (multiple images from n=5-6/ group). *p<0.05, ***p<0.001 PyMT/PBS vs. PyMT/CIS; $^{\#}$p<0.05, $^{\#\#\#}$p<0.001 vs. PyMT/CIS by *unpaired student t-test*. Scales: 50 μm.

Given that the synergistic effects of TZDs are indeed obtained through the decrease of endotrophin-mediated downstream pathways, we predicted that COL6$^{-/-}$ mice would have a reduced benefit from TZD combination therapies on cisplatin sensitivity. To address this point, we assessed the TZD effects on cisplatin treatment in PyMT/COL6$^{-/-}$ mice and compared them to PyMT mice. Indeed, the synergistic effects of TZD on cisplatin were not seen in PyMT/COL6$^{-/-}$ mice, i.e. tumor regression was less affected by TZD combined cisplatin treatment in PyMT/COL6$^{-/-}$ (**Fig. 7A**) and necrotic lesions were barely detectable in the PyMT/COL6$^{-/-}$/TZD/CIS group relative to PyMT/COL6$^{-/-}$ or PyMT/COL6$^{-/-}$/TZD groups (**Fig. 7B-C**). As expected, the TZD effects on EMT suppression were not observed in PyMT/COL6$^{-/-}$ mice (**Fig. 7D-G)**. These results strongly suggest that the synergistic effects of TZDs are critically dependent on the suppression of endotrophin activity. Hence, the endotrophin levels in association with progression towards EMT are critical determinants of the beneficial effects of TZDs on cisplatin treatment.

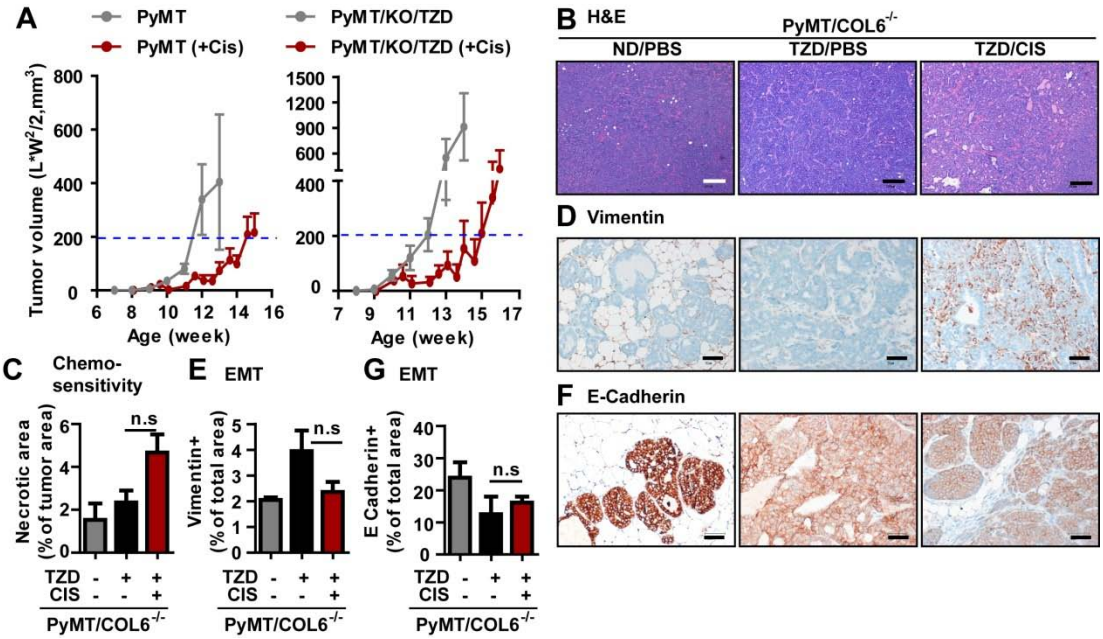


Figure 7. Acquisition of the synergistic effects of TZDs to cisplatin critically depends on the endotrophin levels. Bypassing endotrophin-downstream pathways with COL6$^{-/-}$ mice abolish synergistic effects of TZD in cisplatin sensitivity. PyMT/COL6$^{-/-}$ mice were given TZD (20mg/kg) or ND at 8 weeks of age. Cisplatin (1mg/kg, ip, 2 times/ week) or PBS treatment was initiated in 10 week old mice. Tumor growth (**A**) was determined by caliper measurements, and PyMT littermates were represented as a controls. Data represent mean ± SEM (n=6-8/ group). H&E staining (**B**) and necrotic area quantification (**C**), showing no necrotic area in PyMT/COL6$^{-/-}$ mice following TZD combination with cisplatin treatment. Scales: 200 μm. Vimentin (**D**-**E**) and E-cadherin (**F**-**G**) staining quantification, showing no changes of the EMT status in PyMT/COL6$^{-/-}$ mice by TZD combined cisplatin treatment compared to PBS groups. Data represent mean ± SEM (multiple images from n=5-6/ group). p=n.s (no significant) vs. PyMT/COL6$^{-/-}$/TZD by *unpaired student t-test*.

Task 3. Characterize the effects of VIα3-C5 neutralizing monoclonal antibodies in combination with TZDs to demonstrate synergistic enhancement of susceptibility to cisplatin treatment for breast cancer (18-36 months): ***Completed in year 3***

3a. Tumor tissues or MET cells (mammary epithelial cells) from PyMT/COL6$^{+/+}$ mice will be implanted into WT mice.

3b. Perform the treatment with TZD and VIα3-C5 monoclonal antibodies in combination with cisplatin

3c. Collect samples and analyze the tumor progression and cisplatin susceptibility (24-36 months).

As a last step, I determined therapeutic potential of endotrophin neutralizing antibodies (10B6) that we have previously described on cisplatin sensitivity. Tumor pieces taken from PyMT mice were implanted into wild-type mice and treated with cisplatin alone or in combination with either TZD or 10B6 once the tumor volume reached 100 mm^3. Tumor regression was monitored until 2-months post implantation. We see that both TZD and 10B6 treatment efficiently sensitized the tumors to cisplatin therapeutics (**Fig. 8A**), suggesting that endotrophin neutralization is a powerful approach to chemotherapy sensitization.

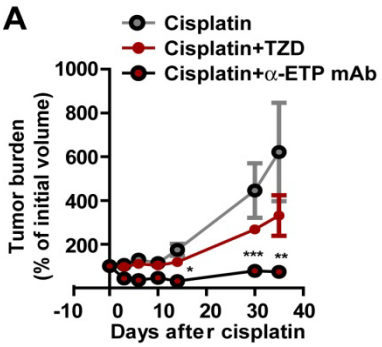


Figure 8. Neutralizing endotrophin activities with monoclonal antibodies sensitizes tumors to cisplatin treatment. A. Pieces of tumors from PyMT mice were implanted into wild-type hosts. Tumor-bearing mice were given cisplatin (1mg/kg, ip, every 5 days) or PBS, combined with either TZD (20mg/kg) or anti-endotrophin monoclonal antibodies (100μg/ mouse, once a week) for tumor progression. Tumor volumes were

determined by caliper measurements. Data represent mean ± SEM (n=5/ group). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. Cisplatin by *2-way ANOVA*.

***Specific Aim5*. Verify the relevance of VIα3-C5 levels in clinical samples (6-36 months).**

Task 1. Verify the levels of VIα3-C5 in breast cancer mouse subjects (6-12 months).
1a. Measure the VIα3-C5 protein levels in MMTV-PyMT mice: ***Completed in year 1***
1b. Measure the circulating levels of VIα3-C5 (6-12 months): Development of ELISA method to detect circulating VIa3-C5 proteins in the serum samples of mouse and human breast cancer subjects. At least 5 samples in each group will be used in this experiment.

We have been developing the sandwich-ELISA method using COL6A3-C5 specific rat monoclonal antibody and rabbit polyclonal antibody as a capturing and detection antibodies. We expect to have optimal conditions shortly for ELISAs to measure the COL6A3-C5 in circulation. Initially we used an anti-mouse COL6A3-C5 polyclonal Ab which seems to be less efficient to detect human C5. Thus, we have generated a novel **anti-human C5** rabbit polyclonal antibody which is specific for human. We will set up the optimal conditions for the ELISA measuring human C5 levels in circulation.

Task 2. Verify the levels of VIα3-C5 in breast cancer human subjects (12-36 months): ***Completed in year 3***
2a. Collect human tissue samples from UTSW cancer tissue bank and analyze the levels of VIα3-C5 by immunohistochemistry using VIa3-C5 antibodies (12-18 months): ***completed***
2b. Measure the VIα3-C5 levels in both serum and breast tumor samples of breast cancer patients at UTSW Medical Center (24-36 months)
2c. Measure the VIα3-C5 levels in human samples after platinum-based chemotherapy in combination with TZDs to characterize VIα3-C5 effects on drug resistance (24-36 months).

We have determined COL6A3-C5 levels in human breast cancer patients with anti-human ETP polyclonal antibody. Endotrophin is highly expressed in tumor tissues compared to benign tissues, thus endotrophin is a promising cancer biomarker. Our collaborator Dr. David Euhus at UTSW Medical Center will play a critical role in spearheading and facilitating these efforts. So far, the human samples are not ready to test.

KEY RESEARCH ACCOMPLISHMENTS

Year 1

- **Established COL6A3-C5 transgenic mice under the control of MMTV promoter: low and high expressors identified.**
- **Generated both polyclonal and monoclonal antibodies against COL6A3-C5 domain.**
- **Determined the COL6A3-C5 protein levels in both human and mouse mammary tumor tissues by immunohistochemistry with COL6A3-C5 specific polyclonal antibodies.**
- **Determined anti-apoptotic and pro-mitotic activities of COL6A3-C5 protein *in vivo*.**
- **Determined the ratio of tumor progression and pulmonary metastasis in COL6A3-C5 transgenic mice in the background of MMTV-PyMT (PyMT/COL6A3-C5) compared to control littermates *in vivo*.**
- **cDNA microarray analysis successfully performed with tumor tissues from PyMT/COL6A3-C5 and PyMT mice.**
- **Generation of MMTV-F635 (infrared fluorescence protein) transgenic mice for *in vivo* imaging of mammary tumor progression successfully accomplished.**
- **Optimized the protocols for immunotherapy with COL6A3-C5 monoclonal antibodies.**
- **Investigated the cisplatin susceptibility in combination with TZD to examine the effects of COL6 on drug resistance (large cohort experiments).**

Year 2

- **Determined the tumor burden at the whole body level with the MMTV-FP635 transgenic mice through *in vivo* imaging.**
- **Determined the cisplatin susceptibility of PyMT/COL6$^{-/-}$ mice compared to tumor-size matched PyMT/COL6$^{+/+}$ mice.**
- **Determined the cisplatin susceptibility in combination with TZD to examine the effects of COL6 on drug resistance (large cohort experiments).**
- **Established the protocols for primary culture of cancer cells originated from MMTV-PyMT tumor tissues.**
- **Introduced cancer cell implantation methods into cisplatin treatment protocols in combination with TZD to examine the effects of COL6 on drug resistance.**
- **Generated a novel anti-human COL6A3-C5 rabbit polyclonal antibody, which is more specific for human C5 as determined by immunohistochemistry.**

Year 3

- **Determined the cisplatin sensitivity with either cancer cells or tumor piece allograft models.**
- **Examined the functional roles of COL6A3-C5 on mammary tumor progression.**
- **Determined the impacts of COL6A3-C5 on cisplatin resistance.**
- **Modeling the chemo-responsiveness in association with COL6A3-C5 levels and EMT.**
- **Determined the therapeutic potential of neutralizing monoclonal antibodies against COL6A3-C5 combined with cisplatin treatment.**

REPORTABLE OUTCOMES

Reportable outcomes that have resulted from this research project:

- Manuscripts:
  - The Adipocyte-Derived Factor Endotrophin Links Obesity with Malignant Tumor Progression

  Park J. and P.E. Scherer, (2012) ***Journal of Clinical Investigation, in press***

- - Paracrine and Endocrine Effects of Adipose Tissue on Cancer Development and Progression.

  Park, J., Euhus, D. and P.E. Scherer, (2011) ***Endocrine Reviews, 32(4):550-70***.

- - Leptin and cancer: from cancer stem cells to metastasis.

  Park J. and P.E. Scherer, (2011) ***Endocrine-Related Cancer: 18(4):C25-9***

- Abstracts:
  - 2011 UKC Conference, Park City, Utah.
  - 2012 Keystone Meeting, Santa fe, New Maxico.

- Oral Presentations:
  - Touchstone Diabetes Center Seminar Series at UTSW.
  - 2011 Era of Hope Conference, concurrent symposium session, Orlando, Florida.
  - 2011 Korean-American Bio-medical Scientists Symposium (KABMS), Houston, USA

CONCLUSION

I have characterized the properties of the adipocyte-derived type 6 collagen-alpha3-C5 domain (COL6A3-C5, referred as endotrophin) on mammary tumor progression and metastasis *in vivo*. I established transgenic mice that express endotrophin under the control of the MMTV promoter. We bred these mice with MMTV-PyMT mice to investigate endotrophin effects on mammary tumor progression and metastasis. My results indicate that **endotrophin is a potent new mitogen**, **stimulating tumor cell proliferation** as well as **enhancing pulmonary metastasis *in vivo***. I will pursue these observations further to quantify the properties of cancer cells with respect to motility and invasion regarding metastasis with primary cultured mammary epithelial tumor cells (MET cells) from PyMT/endotrophin and PyMT mice. To identify the molecular mechanisms on endotrophin mediated cell proliferation and survival, we performed cDNA microarray analysis with tumor tissues from 12-week old PyMT/endotrophin and PyMT mice. Based on our microarray results, 45 % of genes significantly modulated by endotrophin are related to phosphorylation, suggesting that endotrophin is a signaling molecule stimulating a kinase (or a phosphatase) activity. We currently analyze the microarray data in further detail and will focus on several target molecules regulated by endotrophin-mediated signaling pathways.

For the therapeutic purposes, we generated endotrophin specific monoclonal antibodies and have tested these on a mouse mammary tumor model, the MMTV-PyMT mice. Our results suggest that endotrophin monoclonal antibodies **have potent protective effects on mammary tumor progression**.

I have investigated cisplatin resistance of COL6 knock-out mice in the background of MMTV-PyMT mice and compared that to control MMTV-PyMT mice. PyMT/COL6$^{-/-}$ mice were more susceptible to cisplatin treatment relative to PyMT as judged by tumor volume over the course of cisplatin treatment. However, I noticed that it is challenge to determine the drug resistance of MMTV-PyMT mice, because the tumor growth in this model is so aggressive. To address these questions under better conditions, we used tumor cell implants with primary cultured mammary epithelial tumor cells from PyMT/COL6$^{-/-}$ vs. PyMT mice. COL6$^{-/-}$ cancer cells fail to survive in the wild-type hosts; however, endotrophin reconstitution into COL6$^{-/-}$ reversed tumor growth and this increase was sufficient to induce cisplatin resistance.

In summary, I have employed a rodent model for a chemotherapeutic tumor response, and demonstrated that the endotrophin-mediated induction of the EMT results in chemo-resistance. Furthermore, we highlighted that the synergistic effects of TZDs on cisplatin-based therapies are mediated through the suppression of this pathway. Bypassing the endotrophin-induced EMT in the context of COL6$^{-/-}$ mice diminished the TZDs-mediated synergistic effects on cisplatin therapeutics and resulted in a poor response. These results provide a direct explanation for previous correlative studies that demonstrated poor responses to platinum-based chemotherapy in tumors expressing high levels of COL6, and also suggest endotrophin levels as a promising predictive marker to decide if a TZD combination should be initiated along with a platinum-based therapeutic approach.

I plan to measure endotrophin levels in human cancer patient serum samples with the ELISA; unfortunately, the samples are not ready at this time point. If I can detect the endotrophin level in human samples, this has the potential to be a useful therapeutic marker for cancer patients as outlined in our original rationale.

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